

5-19-94  
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MRID No. 427182-03  
429951-01

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Methyl Anthranilate.  
Shaughnessey Number: 128725.
- 2. **TEST MATERIAL:** Methyl anthranilate; CAS No. 134-20-3;  
Batch/Lot No. 387.
- 3. **STUDY TYPE:** 72-2. Freshwater Invertebrate Toxicity Test.  
Species Tested: *Daphnia magna*.
- 4. **CITATION:** Clark, L., C. Goulden, and J.N. McNair. 1992.  
Daphnid Acute Toxicity Test. Study performed by Academy of  
Natural Sciences of Philadelphia, Philadelphia, PA.  
Submitted by PMC Specialties Group. EPA MRID No. 427182-03.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.  
Associate Scientist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: *Rosemary Graham Mora*

Date: 20 Sept 1993

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.  
Senior Scientist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: *Pim Kosalwat* 5/19/94

Signature: P. Kosalwat

Date: 9/20/93

Henry T. Craven, M.S.  
Supervisor, EEB/EFED  
USEPA

Signature:

Date:

- 7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for an acute toxicity study using freshwater invertebrates. The percentage active ingredient of the test material was not reported. Based on mean measured concentrations, the 48-hour EC<sub>50</sub> was 18.2 mg/l which classifies methyl anthranilate as slightly toxic to *Daphnia magna*. The 48-hour NOEC was 5.5 mg/l.
- 8. **RECOMMENDATIONS:**
- 9. **BACKGROUND:**
- 10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.
- 11. **MATERIALS AND METHODS:**

- A. **Test Animals:** *Daphnia magna* were obtained from in-house cultures which were originally received from EPA-Duluth.
- B. **Test System:** The test was conducted under static conditions. The test chambers were 220-ml Anchor-Hocking plastic cups containing 200 ml of test solution. The dilution water was obtained from the Round Valley Reservoir, NJ, and had an alkalinity of 39 mg/l, a hardness of 44 mg/l, and a conductivity of 1.25  $\mu$ mho.

The test temperature was  $20 \pm 1^\circ\text{C}$ . Test solutions were not aerated during the test. The photoperiod was 16 hours of light and 8 hours of darkness.

Ethanol was used as carrier of the test material at a maximum concentration of 50  $\mu$ l/l.

- C. **Dosage:** Forty-eight-hour static test. Based on the results of a range-finding test, five nominal test concentrations (3.125, 6.25, 12.5, 25, and 50 mg/l) were selected for this test. In addition, a dilution water control and a solvent control were included.
- D. **Design:** Five daphnids (1-24 hours old) were randomly selected and transferred to each of four replicate vessels per treatment. The daphnids were not fed during the test. Mortality (immobility) and abnormal behavior were assessed at 3, 6, 12, 24, and 48 hours during the test.

Temperature, pH, and dissolved oxygen concentration (DO) were measured in each chamber at test initiation, 24 hours, and at test termination.

Samples were collected from each treatment at test initiation and from each vessel at test termination for analysis using high pressure liquid chromatography.

- E. **Statistics:** The  $EC_{50}$  and  $LC_{50}$  values were calculated by trimmed Spearman-Kärber analysis.

12. **REPORTED RESULTS:** Mean measured concentrations were 2.4, 5.5, 11.2, 22.2, and 44.4 mg/l (Table 1, attached).

By 48 hours, no sublethal effects or mortality was observed in the controls or the two lowest test concentrations (Table 2, attached). Mortality and sublethal effects in the

remaining concentrations ranged from 0 to 100% and 0 to 65%, respectively.

Based on initial measured concentrations, the 48-hour EC<sub>50</sub> (95% confidence interval [C.I.]) was 19.3 (16.7-22.4) mg/l and the 48-hour LC<sub>50</sub> was 31.3 (28.5-34.3) mg/l.

During the test, the solutions had a pH of 7.7-8.0, a DO of 7.7-8.3, and a temperature of 19.2-20.9°C.

**13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**  
No conclusions were presented by the author.

The GLP compliance statement, which was written by the study submitter, stated that the submitter did not know whether the study was conducted in accordance with 40 CFR Part 160. A quality assurance statement were included in the report indicating the test was performed according to Good Laboratory Practice Standards (40 CFR 292).

**14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

**A. Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following deviations:

The percentage active ingredient and physical characteristics of the test material were not reported.

The concentration of solvent in the solvent control was not reported.

Plastic cups were used as test containers. Glass or stainless steel containers are recommended. Plastics are known to adsorb organic pesticides. However, it does not appear to be the case for this test since measured concentrations were similar to nominal values.

Dilution water was obtained from a reservoir which is surface water, probably subjected to contamination. There is no report whether the water had been analyzed for contaminants before use in the test.

The system used to maintain the test temperature was not reported.

**B. Statistical Analysis:** The reviewer calculated the 48-hour EC<sub>50</sub> and LC<sub>50</sub> values using mean measured concentrations and EPA's Toxanal computer program

(printouts, attached). Based on the number of dead and affected test organisms, the 48-hour EC<sub>50</sub> was 18.2 mg/l (95% C.I. of 15.4-21.3 mg/l). Based on the number of dead organisms only, the 48-hour LC<sub>50</sub> was 29.1 mg/l (95% C.I. of 22.2-44.4 mg/l).

C. Discussion/Results: This study is scientifically sound but does not meet the guideline requirements for an acute toxicity study using freshwater invertebrates. The percentage active ingredient of the test material was not reported. Based on mean measured concentrations, the 48-hour EC<sub>50</sub> was 18.2 mg/l which classifies methyl anthranilate as slightly toxic to *Daphnia magna*. The 48-hour NOEC was 5.5 mg/l.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: The percentage active ingredient of the test material was not reported.

(3) Repairability: Yes; this study may be upgraded to "core" upon submission of the percent active ingredient for this test material.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes; 15 September 1993.

TABLE 1

DEFINITIVE TEST: METHYL ANTHRANILATE  
 : Nominal and Measured Concentrations

Time	Rep.	Concentration (ppm)				
00 h (nominal)		3.13	6.26	12.5	25.0	50.0
00 h (measured)		3.10	6.20	11.9	23.8	47.2
48 h (measured)	1	2.71	5.30	11.0	21.4	43.1
	2	1.90	5.40	11.1	21.8	44.1
	3	2.60	5.30	10.9	22.3	44.5
	4	1.70	5.40	11.1	21.8	43.3
	Ave.	2.23	5.35	11.0	21.8	43.8

Mean Measured  
 Conc.      2.4    5.52    11.2    22.2    44.4

Table 2

DEFINITIVE TEST: METHYL ANTHRANILATE  
Survival Data

Conc. (ppm)	Rep.	Exposure time (hours)																							
		0				3				6				12				24				48			
		A	A	S	D	A	A	S	D	A	A	S	D	A	A	S	D	A	A	S	D				
SC	1	5	5			5				5				5				5							
	2	5	5			5				5				5				5							
	3	5	5			5				5				5				5							
	4	5	5			5				5				5				5							
	Total	20	20	0	0	20	0	0	0	20	0	0	0	20	0	0	0	20	0	0	0				
%	100	100	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0					
C	1	5	5			5				5				5				5							
	2	5	5			5				5				5				5							
	3	5	5			5				5				5				5							
	4	5	5			5				5				5				5							
	Total	20	20	0	0	20	0	0	0	20	0	0	0	20	0	0	0	20	0	0	0				
%	100	100	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0					
3.125	1	5	5			5				5				5				5							
	2	5	5			5				5				5				5							
	3	5	5			5				5				5				5							
	4	5	5			5				5				5				5							
	Total	20	20	0	0	20	0	0	0	20	0	0	0	20	0	0	0	20	0	0	0				
%	100	100	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0					
6.25	1	5	5			5				5				5				5							
	2	5	5			5				5				5				5							
	3	5	5			5				5				5				5							
	4	5	5			5				5				5				5							
	Total	20	20	0	0	20	0	0	0	20	0	0	0	20	0	0	0	20	0	0	0				
%	100	100	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0					
12.5	1	5	5			5				5				5				5							
	2	5	5			5				5				5				5							
	3	5	5			5				5				5				5							
	4	5	5			5				5				5				5			1				
	Total	20	20	0	0	20	0	0	0	20	0	0	0	20	0	0	0	19	1	0	0				
%	100	100	0	0	100	0	0	0	100	0	0	0	100	0	0	0	95	5	0	0					
25	1	5	5			5				5				3	2			1	4						
	2	5	5			4		1		1	3	1			4	1		2	2	1					
	3	5	5			3	2			2	3			1	4			4	4	1					
	4	5	5			4	1			5				4	1			2	3						
	Total	20	20	0	0	16	3	1		13	6	1		8	11	1		5	13	2					
%	100	100	0	0	80	15	5		65	30	5		40	55	5		25	65	10						
50	1	5			5					5							5				5				
	2	5			5					5							5				5				
	3	5			5					5							5				5				
	4	5			5					5							5				5				
	Total	20	0	0	20	0	0	20	0	0	20	0	0	20	0	0	20	0	0	20	0	20			
%	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	100				

SC = Solvent Control  
 C = Control  
 A = Alive and unaffected  
 S = Alive but affected  
 D = Dead

Rosemary Graham Mora MA Daphnia magna EC<sub>50</sub>

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
44.4	20	20	100	9.536742E-05
22.2	20	15	75	2.069473
11.2	20	1	5	2.002716E-03
5.5	20	0	0	9.536742E-05
2.4	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 11.2 AND 22.2 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 17.78584

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	5.135006E-02	18.06797	15.14336	21.88459

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
10	.1969144	1	.9989438

SLOPE = 7.909567  
95 PERCENT CONFIDENCE LIMITS = 4.399693 AND 11.41944

LC50 = 18.16993  
95 PERCENT CONFIDENCE LIMITS = 15.38183 AND 21.30491

LC10 = 12.55417  
95 PERCENT CONFIDENCE LIMITS = 8.740559 AND 14.93062

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Rosemary Graham Mora MA Daphnia magna *LC50*  
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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
44.4	20	20	100	9.536742E-05
22.2	20	2	10	2.012253E-02
11.2	20	0	0	9.536742E-05
5.5	20	0	0	9.536742E-05
2.4	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 22.2 AND 44.4 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 29.12311

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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Ecological Effects Branch One-Liner Data Entry Form

Chemical Methyl Anthranilate Shaughnessy No. 128725 Pesticide Use

INVERTEBRATE ACUTE TOXICITY	% AI	EC <sub>50</sub> (95%CL) SLOPE	HRS/TYPE	NOEC	STUDY/REVIEW DATES	MRID/CATEGORY	LAB	RC
1. <i>Daphnia magna</i>	NR	18.2 (15.4- 21.3) mg/l 7.9	96 h	5.5 mg/l	1992/1993	42718203 <i>Supplemental</i>	ANS	RGM
2.								
3.								
4.								
5.								
6.								
7.								
CHRONIC TOX.	% AI	MATC LC <sub>50</sub>	DAYS	AFFECTED PARA.	STUDY/REVIEW DATES	MRID/CATEGORY	LAB	RC
1.								
2.								

COMMENTS: ANC=Academy of Natural Sciences of Philadelphia. Based on mean measured concentrations. NR=Not Reported